OBSERVATIONS ON THE PHYSIOLOGICAL REACTIONS OF THE DUCTUS ARTERIOSUS

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In a previous paper (Kennedy and Clark, 1941), we have pointed out that the ductus arteriosus is a blood vessel having special characteristics which distinguish it from other large vessels, both in its structure and in its physiological reactions. The normal structural characteristics and the changes following normal closure were presented, and it was pointed out that patency of the ductus arteriosus, one of the common forms of congenital heart disease, may be due to the failure of a physiological mechanism rather than to an embryological malformation. The present paper is concerned with physiological reactions of the ductus.

We have already presented evidence to show that the closure of the ductus arteriosus is a process having two distinct phases. The first is an immediate reaction taking place a few minutes after birth and is essentially a contraction of the muscular wall of the ductus (fig. 1). In some species there has been described a flap-like valve (Hamilton, Woodbury and Woods, 1937) which may aid in closure by obstructing the flow of blood from the aorta into the ductus, but this latter mechanism does not appear to be a factor in the guinea-pig fetus, the animal forming the basis of our study (Harmon and Herbertson, 1938). The second phase of closure is much slower than the first and involves histological closure of the lumen and the replacement of the muscular elements in the wall by fibrous connective tissue resulting in the conversion of the ductus into the ligamentum arteriosum.

The ductus seems to have the capacity to close in response to a variety of stimuli, and in this respect its reaction is quite different from that of the aorta and pulmonary artery. We observed that following the onset of breathing in normal guinea pigs closure of the ductus arteriosus occurred within 4 to 10 minutes. After a few experiments it became apparent that closure of the ductus followed artificial inflation of the lungs with air as well as normal breathing. This suggested that the reaction of closure occurred as a reflex response to definite stimuli, and in an effort to throw light on this conception we entered on the present series of experiments.

MATERIALS AND METHOD. We have used more than 90 pregnant guinea pigs and made observations on upwards of 175 fetuses. By using the technique described previously, which involves delivering the fetuses by operation beneath
the surface of a warm saline bath and leaving the placental circulation and cord vessels intact, prolonged observations can be made on each fetus and the various reactions of the ductus can be studied. The spinal cords of the mother guinea pigs were sectioned in the lower thoracic region under ether anesthesia in preparation for all experiments except a few and where any of the latter were used in the experiments given as examples, special mention is made of it in the protocols. Most of the fetuses used were near term. In some animals the day of fertilization and length of pregnancy were accurately known. All the fetuses used were weighed and the crown-rump lengths measured. These were compared with the weights and lengths appearing in published data on normal guinea pigs. According to Draper (1920) and Ibsen (1928) guinea pig fetuses at term have an average weight of 80 to 85 grams and are about 10 cm. in length. The individual weights vary with the size of the litter from 70 grams with 6 fetuses to 110 grams with 1 fetus (Ibsen, 1928). The gestation period for guinea pigs has an average duration of 68 days (Ibsen, 1928).

Fig. 1. Drawing showing the appearance of the ductus arteriosus of fetal guinea pig open and closed. In the figure the arch of the aorta is shown in the upper part of the opening of the chest, with the pulmonary artery just below it, apparently continuous (by way of the ductus) with the descending aorta (cf. fig. 2). The ductus in the larger right hand figure closed following artificial inflation of the lungs with air. The lighter color of the lung margin (being retracted below the heart) following inflation is apparent. The smaller figure to the right gives the appearance of the fetus with opening in chest wall. In each figure the tracheal cannula is shown.
RESULTS. In fetuses near term it was found that the ductus will close following several different types of stimuli (fig. 2) and it will subsequently open again if the stimulus producing the closure is discontinued. This sequence of closing and opening can be repeated at will. For example, intermittent rhythmic inflation of the lungs with puffs of air through a tracheal cannula at a rate of about 50 per minute (to simulate breathing) caused the ductus to close 7 times consecutively in the same animal, each closure being followed by opening, as shown by the following protocol. Protocol 1. Weight of fetus 93 grams; length 11.4 cm. (crown-rump), selected from a group of such experiments.

Fetus removed from uterus 2:08 p.m. Chest opened and ductus arteriosus observed to be open. 2:11 cannula inserted into trachea.

<table>
<thead>
<tr>
<th>Ductus open</th>
<th>Times at which successive inflations were begun</th>
<th>Period of inflation required for closure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. 2:14</td>
<td>2 min., 30 sec.</td>
</tr>
<tr>
<td></td>
<td>2. 2:23\frac{1}{2}</td>
<td>1 min.</td>
</tr>
<tr>
<td></td>
<td>3. 2:36\frac{1}{2}</td>
<td>3 min., 30 sec.</td>
</tr>
<tr>
<td></td>
<td>4. 2:47</td>
<td>2 min., 30 sec.</td>
</tr>
<tr>
<td></td>
<td>5. 2:56</td>
<td>2 min., 30 sec.</td>
</tr>
<tr>
<td></td>
<td>6. 3:05</td>
<td>3 min.</td>
</tr>
<tr>
<td></td>
<td>7. 3:14\frac{1}{2}</td>
<td>2 min., 30 sec.</td>
</tr>
</tbody>
</table>

The ductus arteriosus was allowed to open following each closure.

In lively guinea-pig fetuses near term, this type of inflation of the lungs with air was almost invariably followed by closure of the ductus. It was therefore used as a method for producing closure and various experiments were designed to interrupt the mechanism operating in the hope of determining the underlying causes of closure of the ductus. With the possibility that closure following pulmonary inflation was dependent upon a neurological pathway a series of experiments was performed in which portions of the nervous system were destroyed with the idea of interrupting a reflex mechanism.

*Experimental search for reflex paths.* Subsequent to each of the following procedures the ductus was successfully closed by intermittent inflation of the lungs through a tracheal cannula:

1. Tissue dissected off anterior surface of ductus (including left vagus and phrenic nerves).
2. Bilateral section of vagus nerves.
   b. Bilateral removal of stellate ganglia and section of both vagus nerves.
4. a. Bilateral ligation of carotid arteries below bifurcation.
   b. Bilateral removal of carotid arteries including their bifurcation.
   c. Bilateral removal of carotid arteries including their bifurcation plus bilateral section of vagus nerves.
5. Ligature pulled very tightly around all vessels, nerves and other structures of mediastinum, cephalic to aortic arch (except trachea).
   a. Cross section of cord at level of 3rd cervical; 6 thoracic; 9th thoracic segments. (Some destruction of cord on each side of section.)
   b. Destruction of large portions of spinal cord; from 2nd cervical to 2nd thoracic segment; 3rd cervical to caudal end; 1st thoracic to 11th thoracic segment.
7. Lesions of spinal cord plus other structures.
   a. Spinal cord destroyed (T1 to T10) and both vagus nerves cut.
   b. Spinal cord destroyed (C2 to caudal end), all mediastinal structures cephalic to
      aortic arch (except trachea) tied with ligature and both vagus nerves cut.
   c. Entire spinal cord and medulla destroyed, both stellate ganglia removed, both
      vagus nerves cut.

Note: The procedures listed were carried out on more than one fetus. For example, closure of the ductus following inflation was observed in eight animals after bilateral vagus nerve section; in four after bilateral carotid removal; in eight after destruction of the spinal cord; in three after bilateral removal of the stellate ganglion, etc.

The above procedures, in which all known neurological pathways between central nervous system and region of the ductus were interrupted, failed to prevent closure of the ductus following inflation of the lungs with air. The only possible neurological mechanism which might have remained operative would be

1 Vinylite.
a reflex dependent upon local neurons but there is not supporting evidence for
the presence of such a local reflex.

The problem of the mechanism of closure was also approached by a different
type of experiment. Various structures were stimulated with an electric current
of low voltage (60 cycle sine-wave) and the effect on the ductus noted. We have
stimulated in this way the left vagus nerve, right vagus nerve, left cervical
sympathetic, the left phrenic nerve, the left stellate ganglion, the left splanchnic
ergive without causing any noticeable change in the ductus.

It appears from the above experiments that a nerve pathway or a neuromuscu-
lar reflex is not essential for closure of the ductus. It also appears that the
ductus will not close following stimulation of certain nerves which are anatomi-
cally closely associated with it.

It may be appropriate to point out here that in experiments of this sort there
are variables present to disturb the accuracy of the results; such, for example,
as interference with the circulation of the fetus, or that of the uterus or placenta,
etc. For this reason one positive experiment in which the ductus closed is worth
more than one in which no closure occurred. At times when experiments similar
to some of those described above as resulting in closure were performed the
ductus remained open. Those in which closure occurred are more significant
because they show that the destructive lesion did not interrupt the mechanism
of closure under the conditions of the experiment.

As previously stated the ductus was observed to close following several types
of stimuli other than artificial inflation of the lungs with air. The various pro-
cedures employed and their effects upon the ductus can best be shown under the
following headings with protocols of a few representative experiments.

1. Normal breathing. Protocol 2. Weight of fetus 88 grams; length 11.2 cm. (crown
rump).

Fetus delivered by operation 4:29. Umbilical cord intact. Spontaneous, vigorous
breathing began at 4:31. Opening of chest begun 4:35 30". Ductus observed to be closed
at 4:35 45".

2. Mechanical or electrical stimulation of ductus. Any dissection in the vicinity of the
ductus which causes tugging on its wall, or gentle pinching of the ductus with small tissue
forceps, or electrical stimulation of its wall is followed by closure. This reaction differs
from the preceding one in that closure following a local stimulus occurs more promptly,
usually within 15 to 30 seconds, and in that it can occur even a short time after death of the
fetus. Similar stimulation of the great arteries of the thorax is not followed by a reaction
similar to that of the ductus but the umbilical vessels constrict promptly at the site of
mechanical stimulation.

3. Artificial inflation of the lungs with oxygen and nitrogen. Protocol 3. The ductus was
allowed to open following each closure.

Closure of the ductus with these three methods (normal breathing, direct mechanical or
electrical stimulation, and artificial inflation of the lungs) has been observed many times.
While artificial inflation failed at times, there was usually observed some factor which might
have affected the normal response, such as failure of either the fetal or maternal circulation,
length of experiment, temperature of bath, size and age of fetus, etc.

Other observations listed below (sections 4 to 7) have not been repeated a sufficient num-
REACTIONS OF THE DUCTUS ARTERIOSUS

<table>
<thead>
<tr>
<th>DUCTUS OPEN.</th>
<th>INFLATION OF LUNGS WITH:</th>
<th>NUMBER MINUTES INFLATION</th>
<th>STATE OF DUCTUS ARTERIOSUS AT END OF INFLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus 1, weight 128 grams; length 12.3 cm.</td>
<td>Oxygen .......................</td>
<td>6</td>
<td>Closed</td>
</tr>
<tr>
<td>Nitrogen .......................</td>
<td>10</td>
<td>Open</td>
<td></td>
</tr>
<tr>
<td>Oxygen .......................</td>
<td>4.5</td>
<td>Closed</td>
<td></td>
</tr>
<tr>
<td>Nitrogen .......................</td>
<td>7</td>
<td>Open</td>
<td></td>
</tr>
<tr>
<td>Oxygen .......................</td>
<td>1.75</td>
<td>Closed</td>
<td></td>
</tr>
</tbody>
</table>

Fetus 2, weight 117 grams; length 11.8 cm.

| Nitrogen ....................... | 15                        | Open                      |
| Oxygen ....................... | 6                         | Closed                    |
| Nitrogen ....................... | 8                         | Open                      |
| Oxygen ....................... | 4.5                       | Closed                    |
| Nitrogen ....................... | 5                         | Open                      |

ber of times to test fully their reliability in closing the ductus but we have observed several valid experiments of each kind.


b. Protocol 5. Weight of fetus 84 grams; length 11 cm.; 0.5 cc. adrenalin chloride 1/10,000 injected into subcutaneous tissue of abdomen. Ductus closed 2 minutes 20 seconds after injection.

5. Mechanical stimulation of carotid sinus. a. Protocol 6. Weight of fetus 72 grams; length 10.2 cm. Left carotid sinus massaged with blunt end of probe. Ductus which had been open, closed after 2 minutes' massage.

b. Protocol 7. Weight of fetus 71 grams; length 10.2 cm. Right carotid sinus massaged with blunt end of probe. Ductus which had been open, closed after 4 minutes 20 seconds' massage.

6. Hemorrhage. Protocol 8. Weight of fetus 70 grams; length 10 cm. Ductus had remained open and under observation for 25 minutes. Left external jugular vein was cut for purpose of causing hemorrhage which was short and brisk. Ductus closed 6 minutes after beginning of hemorrhage.

7. Unexplained. On several occasions we observed closure of the ductus and were not able to relate it definitely to any of the above factors. For example, in about half a dozen experiments after the tracheal cannula was inserted the ductus closed; or after destruction of the spinal cord by thrusting a pipe cleaner into the vertebral canal; or after opening the chest wall with an associated small hemorrhage the ductus closed; or after the position of the fetus was changed suddenly, and occasionally after the mother struggled, the ductus closed. On the whole these unexplained closures were infrequent but they suggest that there may be factors operating to cause closure which we do not understand and do not at present recognize. It may well be that closure which followed some other procedure should be grouped here, or that some of these experiments belong under another heading.

8. Intravenous injection of oxygen. From the evidence that has been presented it is difficult to arrive at a conception of the cause of closure of the ductus which will fit all the facts observed. The most promising observations from the standpoint of a mechanism used at birth are those concerning the different reactions following inflation of the lungs with air, oxygen and nitrogen. According to our experiments, inflation of the lungs with
pure nitrogen does not result in closure of the ductus and it seems that oxygen is an im-
portant constituent of the gas mixture when closure follows inflation of the lungs. If oxy-
genation of the fetal blood causes closure, then oxygen given to the fetus by any route result-
ing in oxygenation of the blood should have the same effect. In order to test this hypothesis
the following experiment was devised. With the usual technique the ductus was visualized
and by means of a small needle and a tuberculin syringe pure oxygen was slowly injected
into the umbilical vein in a series of tiny bubbles. This procedure was promptly followed
by closure of the ductus in four animals. In one other, whose heart was beating irregularly
a partial constriction of the ductus occurred. In two others the technique of injection
failed and the experiment could not be completed. In no one of these animals were the
lungs inflated before the oxygen was injected.

Protocol 9. Weight of fetus 69 grams; length 11.3 cm. Oxygen injected into umbilical
vein very slowly with small needle. The ductus which had been open, closed after 3 minutes
15 seconds, a total of 0.3 cc. of oxygen was injected.

Note: The irritability of the umbilical vessels to needle puncture can be abolished by
first painting the outside of the cord with formalin (10 per cent), then injecting a few tenths
of a cubic centimeter of formalin into the mucous connective tissue of the cord around the
vessels.

DISCUSSION. We have established that the ductus arteriosus is a structure
which can actively close in response to certain stimuli. It responds to local
mechanical stimulation much the same as certain other hollow muscular struc-
tures by contracting. We do not believe that local mechanical stimulation has
an essential rôle in its closure under physiological conditions. Neither does a
neurological mechanism appear essential to closure following artificial inflation
of the lungs. Our findings are at variance with those of Barcroft, Kennedy and
Mason (1938) with respect to the reaction of the ductus following stimulation of
the vagus nerve, but we believe that the present observations have been ade-
quately controlled.

Of the stimuli causing closure of the ductus which we have explored, it seems
likely that under physiological conditions, breathing is the most important. The
actual filling of the lungs by just any gas is not sufficient. From our experi-
ments it appears that oxygen is a necessary component of the gas mixture since
inflation of the lungs with pure nitrogen will not cause closure. Oxygen by vein
will also cause closure without the necessity of accompanying inflation of the
lungs. It is quite possible that many or all of the unexplained closures (see sec.
7) could be due to an increased oxygenation of the fetal blood in response to
painful stimulation, struggling of the mother or fetus, hemorrhage, etc. There
are other possible sources of stimulation which we have not yet explored fully,
such as various natural humoral substances, CO₂, drugs, etc.

Such an influence as that of oxygen on the ductus may have something in com-
mon with the findings of Figge (1934) who demonstrated a definite effect on the
metamorphosis of the aortic arches and gills in larval forms of the salamander by
variations in oxygen tension of their environment.

If this seemingly important relationship of oxygen to the mechanism of closure
of the ductus is true, it offers a practical indication for treatment of new-born in-
fants, especially those which have difficulty in the oxygenation of their blood.
REFERENCES